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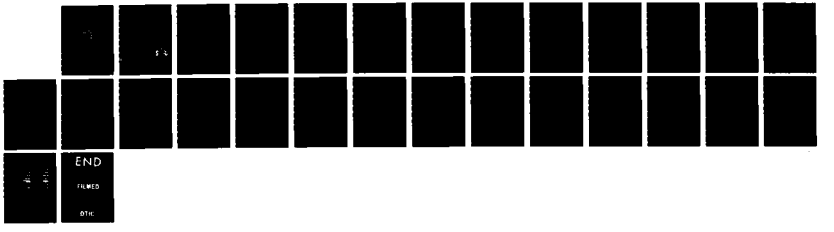

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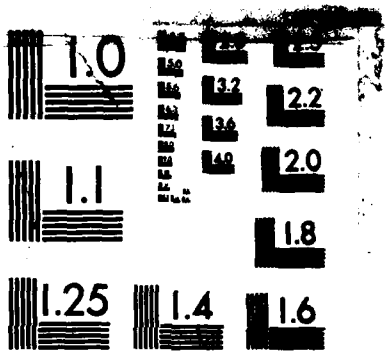

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Aotus trivirgatus are readily infected with HAV following intravenous (IV) inoculation. Since the natural route of infection in man, and probably in monkeys, is the oral route, we undertook further studies to determine the susceptibility of the owl monkey to this route of infection. This paper describes results of our attempts to infect Aotus with HAV via oral and IV inoculation and provides a detailed description of the animal care procedures and surgical techniques utilized in this study.

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Induced Oral Infection of the Owl Monkey (*Aotus trivirgatus*) with Hepatitis A Virus

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Hepatitis A in *Aotus trivirgatus*

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council. USAMRIID is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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Abstract Several species of nonhuman primates have served as animal models for hepatitis A virus (HAV) infection and disease. This study was to determine the suitability of Aotus trivirgatus as an orally induced model for HAV infection, and to reconfirm the owl monkey's susceptibility to the intravenous route of inoculation. Animals were inoculated, either orally or intravenously, with varying concentrations of PA-33 strain of HAV. ALT, AST, and GGTP levels were monitored and liver biopsies performed when values exceeded three standard deviations above individualized mean baseline values. All animals had postinoculation elevations of serum ALT and AST values, shed virus in their feces, and were seropositive to HAV by 60 days after inoculation. Eight of the ten postinoculation biopsy specimens had histologic lesions compatible with acute viral hepatitis. We conclude that the owl monkey is a useful and valuable model for the study of HAV disease.

Chimpanzees (Pan troglodytes) (1,2,3,4), several species of marmosets (Saguinus sp) (5,6,7,8,9,10,11,12,13,14), stump-tailed macaques (Macaca arctoides (15), and owl monkeys (Aotus trivirgatus) (16,17,18) are all susceptible to hepatitis A virus (HAV) infection and have served as valuable animal models. Although the chimpanzee may be the best of these animal models, their endangered status, as well as the elaborate housing facilities required, markedly limit their use. While marmosets are more available, their fragility and diminutive size make them less than ideal subjects for experiments involving multiple liver biopsies or other procedures (14). HAV infection in the stump-tailed macaque has been limited to a single study of three animals (15), and this model has yet to be fully developed. While recognition of the susceptibility of the owl monkey to HAV has come relatively recently (18), this primate species offers several advantages over other susceptible species in terms of availability and size (17).

Aotus trivirgatus are readily infected with HAV following intravenous (IV) inoculation (17). Inoculated monkeys shed virus in their feces, develop elevated serum enzyme values characteristic of liver disease, have liver histopathology indicative of viral hepatitis, and develop antibodies specific to HAV, all similar to infection in man. Since the natural route of infection in man is the oral route, we undertook further studies to determine the susceptibility of the owl monkey to this route of infection. Confirmation of oral susceptibility is needed to determine the suitability of Aotus as an animal model for the evaluation of the degree of protection conferred by candidate HAV vaccines against the natural route of infection. This paper describes results of our attempts to infect Aotus with HAV via oral and IV inoculation, and provides a detailed description of the animal care procedures and surgical techniques utilized in this study. This study was done in conjunction with a formalin-inactivated, HAV vaccine-efficacy study in which

the Aotus model was used. Results of that study will be presented separately (19).

Materials and Methods

Animals: Ten colony-born and reared owl monkeys (Aotus trivirgatus), seronegative to HAV, as determined by a commercial radioimmunoassay[1], and ranging in age from three to six years, were selected for study. Sex and karyotype of each animal is listed in Table 1. All animals were obtained from the Veterinary Resources Branch, Walter Reed Army Institute of Research, (WRAIR), Washington, DC, and were maintained at the Animal Resources Division, United States Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

Clinical Evaluation: Initial evaluation of the 10 animals was done after a 14-day adjustment period. Animals were sedated with ketamine HCl[2] (4mg/kg body wt.) and subjected to a general physical examination, tuberculin skin test, and rectal swab and culture for enteropathogens. Fresh fecal specimens were collected from each animal and submitted for flotation and examination for enteric parasites. Blood samples were drawn via femoral venapuncture and used to evaluate hematological and serum chemistry parameters.

Environment: Animals were housed in a single room in individual stainless steel primate cages, 60 cm wide, 42 cm deep and 60 cm high. All cages were equipped with locking squeeze backs, automatic watering devices and removable stainless steel nesting boxes (20 cm wide, 17 cm deep, 25 cm high). Cage spacing and design were such that the probability of animal-to-animal contact was virtually nil and the possibility of fecal cross contamination extremely low. Humidity and temperature were maintained at 65% and 26°C, respectively. Fluorescent lighting, automatically controlled on a 12 hour light/dark cycle, was subdued by red filters over all but one-half of one fluorescent light tube. Nesting boxes and subdued light sources were special modifications recommended to decrease the intense Aotus reaction

elicited by proximal human activity (20). The white light source, from half of one fluorescent tube, was located at the entrance to the animal room. This served as a work area for diet preparation, food bowl and nest box sanitization, and for blood sampling and minor surgical manipulations. Animal Biosafety Level 2 and Biosafety Level 2 practices (21) were maintained during the infection regimen.

Diet: The primary diet component was a commercial nonhuman primate chow[3] soaked in a solution of commercial orange flavored beverage[4]. Each animal was provided 8-10 soaked biscuits twice daily at approximately 8:00 AM and 3:30 PM. This was supplemented with half an orange, injected with 0.5 ml multi-vitamin drops[5], three times per week. A twice-weekly treat consisted of either 1/4 banana or 1/4 sliced apple. During an initial 10-week acclimatization phase, each animal received 60 g of a fortified high calorie diet supplement[6] twice weekly. Upon acclimatization, this supplement was increased to three times per week.

Experimental Design: During the acclimatization phase, baseline serum chemistries and hematological values were established, and preinoculation liver biopsies were performed. Serum chemistry evaluation and establishment of baseline values consisted of monitoring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transpeptidase (GGTP)[7] levels. The criterion for a significant enzyme elevation was $\geq$ 3 standard deviations above individualized mean baseline values. Monkeys were then inoculated with varying concentrations of HAV by either the IV or oral route. A filtered suspension of Aotus feces containing PA-33 strain HAV which had been shown capable of infecting Aotus (17) was used as inoculum. This material was bacteriologically sterile and contained no other detectable viral agents upon prolonged incubation with OMK-210 cells, an owl monkey kidney cell

line which supports the replication of many agents infectious for Aotus sp. (22). The inoculum was used undiluted (1.0 ml of 0.2% suspension) or diluted 1×10^{-2}, 1×10^{-4}, or 1×10^{-6} in Hanks balanced salt solution, as shown in Table 1.

Following inoculation, serum samples were obtained twice weekly and liver enzyme values monitored for significant elevations above baseline. Fecal samples were collected four times per week, beginning at the start of the infection program and continuing for the duration of the experiment. Each fecal sample was screened for HAV antigen as a 10% suspension in phosphate buffered saline utilizing a solid-phase radioimmunoassay. Specimens generating a sample#negative - control ratio (P/N) greater than 2.1 were considered positive, provided the reaction could be specifically blocked (50% or greater reduction in counts per minute) by postinfection, but not homologous preinfection, reference chimpanzee sera (18). Acute phase liver biopsies were performed when enzyme elevations indicated possible hepatic disease. Post-experiment reintegration into the WRAIR Aotus colony was accomplished after all animals had demonstrable levels of circulating anti-HAV antibody and no evidence of fecal viral shedding for at least 30 days.

Surgery: Liver biopsies were obtained from each animal prior to the inoculation with virus. Following infection, each animal was monitored closely for changes in serum enzyme levels. Development of significantly elevated serum ALT values on two consecutive samples prompted a second liver biopsy. Surgical candidates were fasted 12 hours prior to surgery. Atropine sulfate[8], given at 0.025 mg/kg body wt, was administered 15 minutes prior to surgery. Anesthesia was induced by multiple site intramuscular injection of ketamine HCl, (4 mg/kg body wt.) and xylazine [9] (0.5 mg/kg body wt.). A bland ophthalmic ointment[10] was instilled in each eye once anesthesia was induced.

Abdominal hair was clipped, the surgical site scrubbed with a tamed iodine scrub[11], and the site then swabbed with isopropyl alcohol. Following two additional surgical scrubs, the surgery site was sprayed with a tamed iodine solution[12]. The animal was positioned in dorsal recumbency on a warm water heating pad[13] and surgically draped. A 6 cm midline cranial abdominal skin incision was made using a No. 10 surgical blade. The linea alba was identified and incised 4-5 cm with a fresh surgical blade. The liver was visualized and observed for signs of gross pathological changes prior to selection and exteriorization of an appropriate lobe. A liver wedge was taken using a procedure described elsewhere (23). The liver was closed using compression, simple interrupted sutures of 3-0 polyglycolic acid (PGA)[14] swaged to a tapered half-circle needle. The linea alba was then closed using 3-0 PGA in a simple interrupted pattern. The skin was sutured closed with an interrupted horizontal mattress suture pattern, 3-0 nylon[15] with a swaged on reversed cutting 3/8 circle needle. After closure was complete, the surgical site was liberally sprayed with a topical antibacterial.[16] Animals were maintained immediately postoperatively on a heating pad, under a heating lamp. Rectal temperature and ECG tracings were monitored throughout surgery and recovery. Recovery was considered sufficient for return to caging when the righting reflex was regained. Skin sutures were removed 14 days postoperatively. Preinfection liver biopsy surgical recovery was considered complete when liver enzyme values had returned to baseline values on at least two consecutive blood samplings.

Virus Inoculation: Following ketamine HCl sedation (4 mg/kg body wt.) and surgical preparation of the injection site, 1.0 ml of inoculum was injected, via a 27 ga needle into the lateral saphenous vein. Oral inoculation, also under ketamine sedation, was via a No. 8 French, 38 cm long

feeding tube[17]. The distal end of the tube was lubricated[18] prior to passage through a bite block into the esophagus. Insertion of approximately 20 cm of tubing beyond the bite block resulted in entry of the distal end of the tube into the stomach. Proper positioning of the distal tube tip was confirmed by injecting 1-2 ml sterile water in the feeding tube, aspirating, then checking the aspirant for visual signs of gastric content. One ml of infective inoculum was then injected into the feeding tube, followed by a 3 ml sterile water flush to clear the tube of inoculum. Extubation was accomplished with the flushing syringe remaining connected to the feeding tube.

Results

Biochemical changes associated with HAV infection in Aotus sp: Both monkeys inoculated IV with the undiluted Aotus fecal suspension developed significant elevations of serum ALT (Table 2) at 4 to 18 days postinoculation. These abnormal elevations ranged from 9.0 to 10.7 times base-line value and persisted for 14 to 28 days. AST levels were also significantly elevated during approximately the same period for both animals. A significant rise in GGTP level was noted in one monkey, and occurred somewhat later (25-32 days postinoculation) than initial elevation in ALT and AST levels.

Serum ALT elevations developed in all eight monkeys inoculated orally (Table 2). Compared with IV inoculated monkeys, onset of ALT elevations was somewhat later (18 to 60 days postinoculation) in all orally inoculated animals. Elevations were of equal or greater magnitude than those seen in IV infected animals (up to 19 times preinoculation values) and persisted for 1 to 21 days. One monkey (WR-112) had two periods of elevated ALT values, while another monkey (D-3) had an elevated ALT on only one serum sample (day 48 postinoculation).

Serum AST values were also elevated in orally inoculated monkeys, with magnitudes and days of maximum elevation similar to those of ALT elevations (Table 2). Several animals (C-1-1, D-3, WR-112, and WR-191) had more than one postinoculation episode of elevated AST values, while one animal (WR-208) had an elevated AST on only one serum sample (day 4 postinoculation). Significant elevations of GGTP were noted in four orally inoculated monkeys at 25 to 49 days postinoculation.

Virus excretion and development of anti-HAV: HAV antigen was detected in feces of all monkeys (Table 3). Antigen was detected as early as 5 to 7 days

ıfter IV inoculation and from 13 to 45 days after oral inoculation. The ›resence of viral antigen in feces preceded development of liver enzyme ıbnormalities in one of two IV (Fig. 1-A) and in all orally (Fig. 1-B) ınoculated monkeys. Viral antigen was shed for 8 to 16 days in IV inoculated ınimals, while orally inoculated animals shed virus for 2 to 22 days, ıveraging 12 days.

Sera collected before and after inoculation with virus were tested for anti-HAV (Table 3). Both IV inoculated monkeys were seropositive by 18 days postinoculation. Seropositive orally inoculated monkeys were first seen on day 25 and all were seropositive by 60 days after inoculation.

Histopathological changes associated with HAV inoculations:

Histopathological findings are summarized in Table 4. Preinoculation liver biopsies, which produced no significant hepatic enzyme elevations, were normal with a few minimal changes. Three of 10 monkeys had small numbers of mononuclear cells and a few polymorphonuclear leukocytes in the portal areas. One of these three as well as two others, had scattered foci of lymphocytes, macrophages and polymorphonuclear leukocytes within the hepatic lobule. Necrosis of hepatocytes was not evident in any sections examined from preinoculation biopsies.

Eight of 10 monkeys had histologic lesions compatible with acute viral hepatitis in acute biopsy specimens. Six of 10 had a mild hepatitis, one developed moderate hepatitis and one had minimal hepatitis. Mild to moderate portal infiltration with mononuclear cells, predominantly lymphocytes, some macrophages and a scattering of eosinophils was seen. In the majority of monkeys, inflammation was confined to portal areas and did not breach the limiting plate. Scattered foci of similar inflammatory cells were noted within the hepatic lobule and were present in greater numbers and severity

Figure 1 Course of PA-33 strain HAV infection in two seronegative A. trivirgatus monkeys (L-3 [A] (intravenous) and C-1-1 [B] (oral)). The arrows indicate the day of inoculation. The solid bars represent individual ALT determinations. The open circles represent fecal HAV antigen as determined by radioimmunoassay(RIA); values > 2.1 ratio units are positive. In both monkeys, peak fecal antigen shedding preceded maximal serum enzyme elevations.

Table 4 Histologic features in liver biopsies from HAV inoculated *A. trivirgatus*

Monkey	Route of Inoculation	Dose of HAV	Days Post-Inoculation	Preinoculation biopsy/postinoculation biopsy[a] Portal inflammation	Lobular inflammation	Necrosis	Kupffer Cell hypertrophy
L-3	IV	Undil.	26	0/++	0/+	0/0	+/+
WR-200	IV	Undil.	19	0/++	0/+	0/0	0/+
C-1-1	Oral	Undil.	26	0/+	0/0	0/0	0/0
WR-165	Oral	Undil.	40	+/++	+/+	0/0	0/+
D-3	Oral	1×10^{-2}	60	0/+	0/+	0/0	0/+
WR-163	Oral	1×10^{-2}	40	0/++	0/++	0/0	0/++
WR-112[b]	Oral	1×10^{-4}	19	0/0	+/0	0/0	0/0
			54	0/++	+/+	0/0	0/+
WR-208	Oral	1×10^{-4}	60	+/+	0/0	0/0	0/+
WR-95	Oral	1×10^{-6}	54	0/++	+/+	0/0	0/+
WR-191	Oral	1×10^{-6}	40	+/+++	0/++	0/0	0/++

[a] Interpretation of paired preinoculation and acute-phase liver biopsies: 0, absent; +, minimal; ++, mild; +++, moderate.

[b] Biopsied twice.

TABLE 3 Virological and serological findings in HAV-inoculated *A. trivirgatus*

Monkey	Route of Inoculation	Dose of HAV	Fecal HAV Antigen: Days Present[b]	Fecal HAV Antigen: Maximum P/N[c]	Anti-HAV antibody[a] (%) at (day): Preinoculation	11	18	25	32	39	46	60	139
L-3	IV	Undil.	7-14	20.8(14)	0	0	55	ND[d]	87	ND	ND	96	97
WR-200	IV	Undil.	5-21	42.5(11)	0	0	80	ND	93	ND	ND	96	97
C-1-1	Oral	Undil.	13-27	10.2(18)	0	0	0	51	82	ND	ND	97	97
WR-165	Oral	Undil.	17-32	13.3(24)	0	0	0	40	78	ND	ND	95	97
D-3	Oral	1×10^{-2}	39-41	6.2(39)	0	0	0	0	0	0	94	93	98
WR-163	Oral	1×10^{-2}	27-49	7.3(49)	0	0	0	0	0	82	91	96	97
WR-112	Oral	1×10^{-4}	21-32	21.5(32)	0	0	0	0	44	83	90	96	97
WR-208	Oral	1×10^{-4}	45-56	18.4(45)	0	0	0	0	0	0	0	81	95
WR-95	Oral	1×10^{-6}	32-42	6.1(32)	0	0	0	0	0	0	68	90	96
WR-191	Oral	1×10^{-6}	27-31	4.5(27)	0	0	0	0	31	73	92	95	97

[a] The percent reduction in counts per minute in the Havab® test (> 50% is positive).

[b] Days postinoculation.

[c] P/N, Radioimmunoassay ratio units; all values were reduced by > 50% by the addition of reference anti-HAV serum (18). Day of Maximum P/N).

[d] ND, not done.

Table 2 ALT, AST, and GGTP serum enzyme activities in HAV IV and orally inoculated *A. trivirgatus*

Monkey	Route of Inoculation	Dose of HAV	ALT (U/Liter)			AST (U/Liter)			GGTP (U/Liter)		
			Base line[a]	Maximum[b]	Days Elevated[c]	Base line	Maximum	Days Elevated	Base Line	Maximum	Days Elevated
L-3	IV	Undil.	39.0(16.0)	419(25)	18-32	113.9(38.4)	329(25)	20-25	12.0(5)	---	---
WR-200	IV	Undil.	32.2(14.6)	291(25)	4-32	118.3(37.9)	576(4)	4-25	6.7(3.8)	53(25)	25-32
C-1-1	Oral Oral	Undil.	40.8(18.3)	259(32)	18-39, 67	114.1(31.8)	318(32)	18,32, 67	10.5(3.3)	21	39,67
WR-165	Oral	Undil.	23.2(14.0)	438(32)	32-35	74.5(27.6)	337(32)	32-35	5.9(3.1)	---	---
D-3	Oral	1×10^{-2}	44.5(19.7)	236(48)	48	98.6(28.3)	236(53)	4,53	6.9(3.3)	---	---
WR-163	Oral	1×10^{-2}	39.8(14.0)	392(46)	32-46	136.4(34.5)	422(46)	46	9.7(3.5)	---	---
WR-112	Oral	1×10^{-4}	37.3(12.4)	555(39)	4-11 39-46	107.3(41.0)	473(39)	4-11 39-46	9.5(3.5)	52(46)	39-46
WR-208	Oral	1×10^{-4}	25.2(10.4)	100(67)	60-74	61.8(15.8)	140(4)	4	5.9(3.7)	17	32
WR-95	Oral	1×10^{-6}	33.7(15.3)	613(46)	46-48	77.9(15.1)	387(46)	4,20, 46-49	12.5(3.6)	31	49
WR-191	Oral	1×10^{-6}	35.3(13.9)	312(39)	32-39	88.8(20.8)	344(39)	4,32-35, 39	9.5(3.7)	---	---

[a] Base-line values are the mean of six weekly preinoculation determinations (standard deviations).

[b] (Postinoculation day when maximum values were noted).

[c] Days, postinoculation, when values were elevated more than three standard deviations above the base line.

TABLE 1 Identification and dosage of HAV-inoculated *Aotus trivirgatus*

Monkey Identification				
Number	Sex	Karotype	Inoculum Concentration	Inoculum Route
L-3	Male	III	Undiluted	IV
WR-200	Female	II	Undiluted	IV
C-1-1	Male	III	Undiluted	Oral
WR-165	Male	II x IV (Hybrid)	Undiluted	Oral
D-3	Male	IV	1×10^{-2}	Oral
WR-163	Male	II	1×10^{-2}	Oral
WR-112	Male	III	1×10^{-4}	Oral
WR-208	Male	II	1×10^{-4}	Oral
WR-95	Male	III	1×10^{-6}	Oral
WR-191	Male	Undetermined	1×10^{-6}	Oral

Footnotes

[1] Havab®, Abbott Labs., North Chicago, IL.

[2] Vetalar®, Parke Davis, Morris Plains, NJ.

[3] Monkey Chow 5038®, Ralston Purina Co., St. Louis, MO.

[4] Tang®, General Foods Corp., White Plains, NY.

[5] PolyViSol®, Mead Johnson and Co., Evansville, IN.

[6] Sustecal®, Mead Johnson and Co., Evansville, IN.

[7] Centrifichem-Encorr®, Baker Instruments, Allentown, PA.

[8] Atropine Sulfate Injection, USP, Eli Lilly & Co., Indianapolis, IN.

[9] Rompun®, Haver-Lockhart, Shawnee, KS.

[10] Lacri-Lube®, Allergan Pharm. Inc., Irvine, CA.

[11] Pharmadine Surgical Scrub®, Sherwood Pharm. Co., Mahwah, NJ.

[12] Pharmadine Solution®, Sherwood Pharm. Co., Mahwah, NJ.

[13] Hamilton Aquamatic K Module®, Hamilton Ind., Cincinnati, OH.

[14] Dexon®, Davis & Geck, Inc., Manati, P.R.

[15] Dermalon®, American Cyanamid Co., New York, NY.

[16] Furozolidone®, Veterinary Products Ind., Phoenix, AZ.

[17] Feeding Tube, Dart Industries, Inc., Wallingford, CN.

[18] Surgilube®, Byk-Gulden, Melville, NY.

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Nonhuman primates are the animals susceptible to HAV and therefore used for the study of hepatitis A infection. Future efforts at vaccine development and testing, as well as elucidation of the pathogenesis of HAV-induced hepatitis, will require a substantial number of experimental infections utilizing primate models. The chimpanzee remains the animal model of choice for viral induced hepatitis infections, but its restricted availability severely limits its use. The owl monkey's non-endangered status, ease of husbandry, resilience to manipulation, and susceptibility to HAV via various inoculation routes establishes Aotus trivirgatus as a valuable model for the study of hepatitis A viral infection.

Peak serum enzyme elevations followed shedding of HAV antigen in feces, and occurred at approximately the same time HAV antibody began to develop. The IV inoculated animals showed signs of active HAV infection (i.e., elevated ALT values) slightly earlier than animals orally inoculated with undiluted doses of HAV. This was most likely due to the more rapid hepatic localization of the virus via the IV route of inoculation. Animals orally infected with dilutions of the parent inoculum (10^{-2} - 10^{-6}) exhibited further increased incubation periods and subsequent delayed hepatic enzyme elevation, fecal shedding of virus, and antibody production. Elevation of serum enzymes in WR-208 was delayed by almost two incubation periods. An idiosyncratic variability, inherent with any animal model system, or the possibility of secondary transmission, although remote, could account for this delay.

In the eight monkeys with histologic evidence of hepatitis, inflammatory changes were milder and active necrosis absent, as compared to previous observations (16, 17). These monkeys may have been biopsied beyond peak viral pathological activity, since histologic changes were compatible with a resolving hepatitis. Two monkeys (C-1-1 and WR-208) that did not appear to have histologic evidence of hepatitis may have been biopsied before peak viral activity.

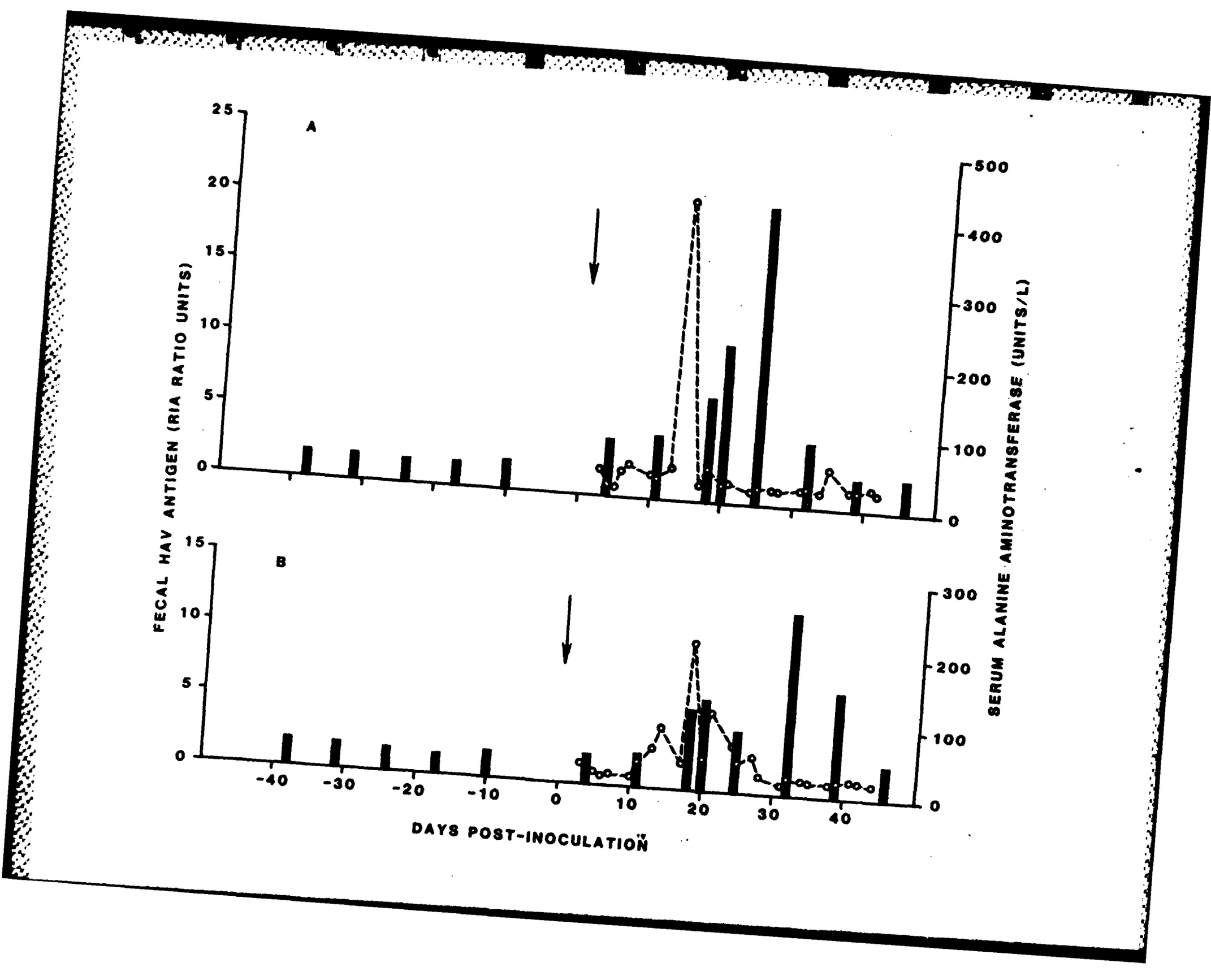
Viral shedding, liver enzyme (ALT) elevation, antibody production, and hepatic histopathologic alteration noted in these infections closely parallel those associated with human hepatitis A (26). These data thus indicate that A. trivirgatus is susceptible to HAV via a more natural route of infection, that of oral inoculation. It complements previous studies (18) and firmly establishes the owl monkey as a useful and valuable model for the study of this human disease.

Discussion

The experimental infections described here confirm previous observations that A. trivirgatus is susceptible to HAV infection via the intravenous route (17), and also confirms previous epidemiologic observations suggesting their susceptibility to oral infection (18). This is important as there is a need for an orally susceptible animal model. Chimpanzees, marmosets, and owl monkeys are the only nonhuman primate species that are reproducibly susceptible to hepatitis A (24). As an animal model for the natural route of infection of this disease, the owl monkey will be valuable in evaluation of hepatitis A virus vaccines. It may also serve as a model in pathogenesis studies directed towards ascertaining the specific sites of initial viral replication and routes of viral dissemination.

Inoculation of A. trivirgatus with HAV was associated with evidence of liver dysfunction and histological changes similar, but milder, than those seen with hepatitis A in humans (25). Serum ALT and AST values were significantly elevated after an incubation period of 4 to 18 days and 18 to 60 days in IV and orally inoculated animals, respectively. The only exception was WR-208, an orally infected animal, which exhibited a single elevation of AST on day four postinoculation. Elevation of GGTP was observed in only one IV inoculated monkey and in only four of eight orally inoculated animals, in contrast to that observed in a previous study in which all infected A. trivirgatus had significant postinoculation elevations of GGTP (17). Although other studies have utilized AST (7,11,14), and serum isocitrate dehydrogenase (7,9,11) activity as indicators of hepatocellular disease and damage in marmosets, results of this and other studies (2) indicate that ALT may be the more reliable enzyme for detecting acute hepatitis due to HAV infection in owl monkeys and chimpanzees.

than observed in control biopsies. Kupffer cells were more prominent in seven of eight monkeys that had histologic characteristics of hepatitis. Neither cholestasis nor active necrosis was observed in any postinoculation biopsy.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